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Bioconversion of synthesis gas to second generation biofuels: A review

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ABSTRACT

The ever increasing concerns over fluctuating price of oil and the possible future supply constrains have reinforced the need for alternative fuel resources. Use of synthesis gas (syngas) to generate fuels through a microbial route would likely be an option to address part of this challenge. Syngas fermentation offers a pathway for sustainable synthesis of fuels and chemicals with lots of advantages over catalytic syngas conversion. This work reviews the available literature on production of second generation biofuels from syngas using various biocatalysts. The potential of syngas fermentation using acetogenic, hydrogenogenic and methanogenic organisms have been surveyed. A vast variety of biofuels and biochemicals including ethanol, acetate, hydrogen, butanol, butyrate, methane and etc. have been produced from gaseous substrates using microbial catalysts. The role of various parameters including medium composition, fermentation pH, trace elements, reducing agents and mass transfer limitations on bioconversion process has been extensively discussed. From this survey of literature it has been deduced that despite years of research and endeavors, fermentation of syngas to biofuels is still a relatively immature technology and long-term view for this potential conversion should be undertaken for future commercial deployments.

Contents

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1. Introduction

For the first time in history one sixth of humanity, which is more than the population of United States, Canada and the European Union, live in hunger [1]. In the world that over one billion people do not have enough to eat, is it ethical to produce fuel from food resources? Whereas, the grain grown by US farmers in 2009 for biofuel production was almost enough to feed 330 million hungers [2].

First generation biofuels which are produced from preliminary food resources such as starch, sugar, vegetable oil and animal fats would increase the global food insecurity. Although production of first generation biofuels like corn-based ethanol in United States, sugarcane-based ethanol in Brazil and canola and sunflower-based biodiesel in Europe will continue as commercially mature technologies, however the increasing criticism over sustainability of these fuels and their competition with food production has turned attentions toward second generation biofuels [3]. The second generation biofuels are derived from non-food lignocellulosic biomass. An overview of potential feedstocks for production of second generation biofuels is depicted in Fig. 1 [3,4]. Generally, these biomass feedstocks are categorized as agricultural residues and by-products, organic wastes and energy crops, which include fast growing woody biomass. Thus, second generation biofuels offer advantages such as utilization of waste residues and use of abandoned lands, especially in rural regions. However, the sustainability of second generation biofuels will be doubtful if they compete with food crops for available land. There is also the concern of excessive withdrawal of agricultural residues for biofuel and bioenergy purposes which could negatively affect the soil fertility and quality due to the decomposing biomass removal [5].

Second generation biofuels are obtained through two different conversion routes of bio-chemical and thermo-chemical approaches. The bio-chemical route involves the conversion of cellulose and hemicellulose components of biomass feedstock to a mixture of fermentable reducing sugars using enzyme or acid hydrolysis; followed by the fermentation of sugars to alcohol, mainly ethanol, using microorganisms. This conversion process relies on utilization of biocatalysts including enzymes and microorganisms. The thermo-chemical conversion process involves the use of gasification or pyrolysis technology under high temperatures to convert the lignocellulosic structure of biomass to an intermediate gas or liquid. Consequently, that intermediate can be transformed to various synthetic biofuels, such as synthetic diesel, aviation fuel and ethanol [6–8]. Many of fuels currently produced from fossil fuels such as refined Fischer–Tropsch liquid (FTL) and methanol could be obtained through the thermo-chemical route [5,9]. Although each of the conversion routes has its advantages or drawbacks, the yield, economics and environmental issues of the two processes are very comparable. So far, no clear commercial/technological predominance has been demonstrated between the achievements and attitudes of the two approaches [6,10,11].

In the bio-chemical conversion process, high selectivity and conversion efficiency is achieved. However, this route requires a critical pretreatment process to alter the structure of biomass to expose the cellulose and hemicellulose for enzymatic hydrolysis; this process along with the high cost of enzymes increases the whole process cost. Conversely, the thermo-chemical conversion route is a robust technology, able to process a wide range of lignocellulosic biomass feedstocks. The major concern in this approach is the cost of a large quantity of biomass which is collected, transported and delivered at the plant gate. This price should be reasonable enough to meet the requirements of commercial biofuel production plants [6].

Generally, there are some key differences between the two conversion approaches. First, in the thermo-chemical conversion the lignin component of biomass is converted to gas along with cellulose and hemicellulose. Whereas, the bio-chemical route can hardly break down the lignin component which constitutes 10–40% of biomass using fermentative or enzymatic reactions to fermentable compounds [12]. Second, ethanol is the major fermentation product obtained through the bio-chemical pathway, while a wide variety of biofuels can be generated from syngas through the thermo-chemical approach. However, the bio-chemical conversion path is probably the most mature process to generate ethanol from the transformation of lignocellulosic biomass [13]. In contrast, the thermo-chemical conversion process has been somehow neglected in the scientific literature.

The thermo-chemical conversion of lignocellulosic biomass integrates the process of biomass gasification and biofuel synthesis as schematically illustrated in Fig. 2. Production of biofuel from syngas is either performed using inorganic or metal-based catalyst known as Fischer–Tropsch (FT) process or microbial catalysts known as syngas fermentation [14,15]. Although the biomass gasification has been extensively studied, however little data are available in the literature regarding its integration with the consequent fermentation process to yield biofuels. This investigation aims to present a detailed review on the utilization of gaseous substrates through a bio-catalytic route to obtain various biofuels. The optimum conditions for various acetogenic, hydrogenogenic and methanogenic organisms to obtain a high product yield have been reported. The effect of various influential parameters on bioconversion process in terms of cell growth, product formation and product ratio has been extensively discussed.

2. Thermo-chemical conversion of biomass

2.1. Biomass gasification

Gasification is the thermo-chemical conversion of carbonaceous feedstock to gaseous products through a partial oxidation process at elevated temperatures. In biomass gasification the lignocellulosic structure of biomass is thermally cracked into carbon monoxide (CO), hydrogen (H_2) and carbon dioxide (CO_2) as the main constituents of syngas and minor amounts of methane (CH_4) and a variety of trace gases. The composition of syngas depends on some parameters such as feedstock properties (ash, moisture, particle size), gasifying agent (air, steam, pure oxygen or a combination of them), type of gasifier (fixed bed, moving bed, fluidized bed, entrained flow) and operation condition (temperature, gasifying agent to fuel ratio, etc.) [16,17].

Although various gasification methods have been implemented to convert the lignocellulosic biomass to syngas, considering the throughput, cost, complexity and efficiency factors, fluidized bed gasification is the most suitable process for large scale gas production [13].

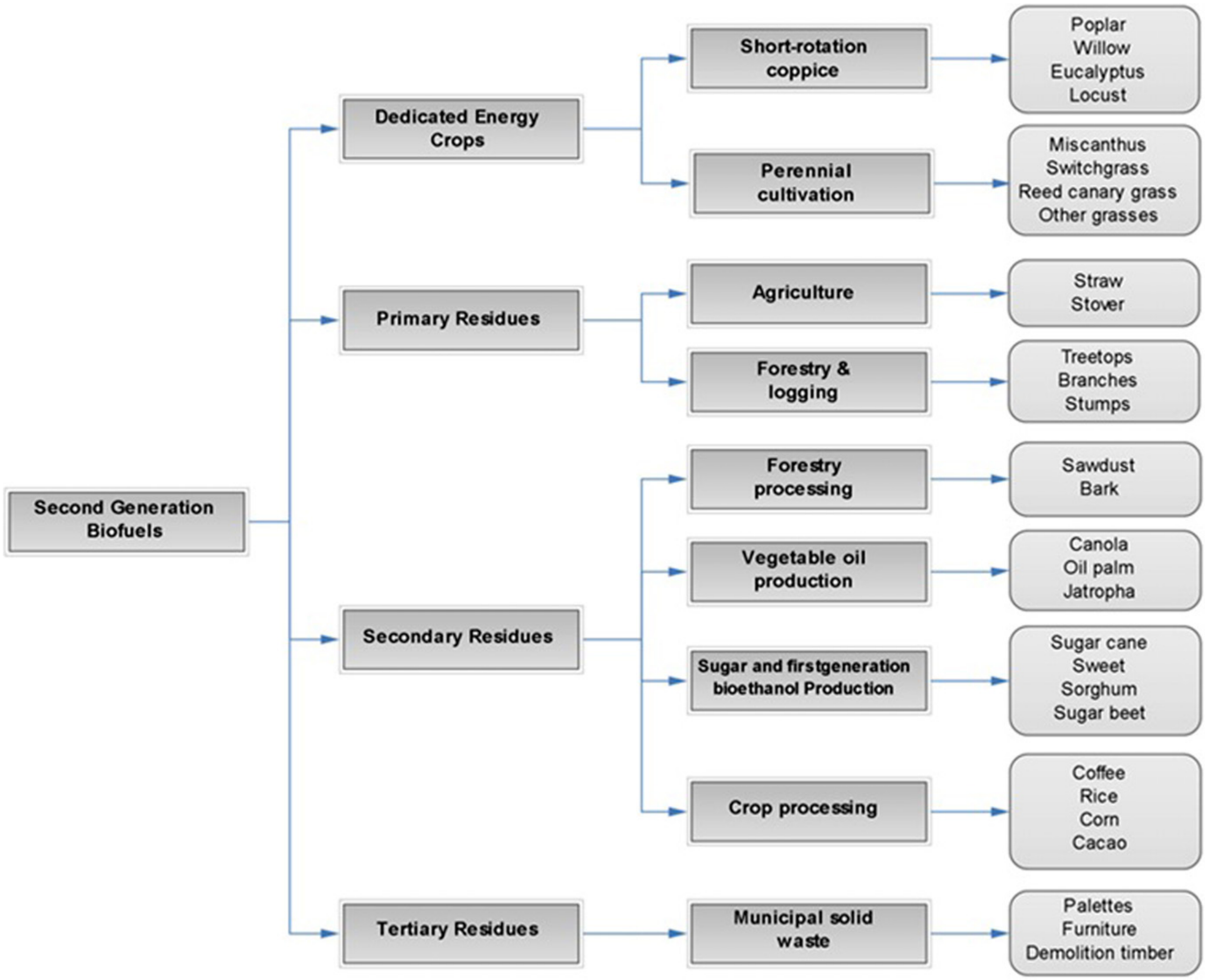


Fig. 1. An overview of potential feedstocks for production of second generation biofuels.

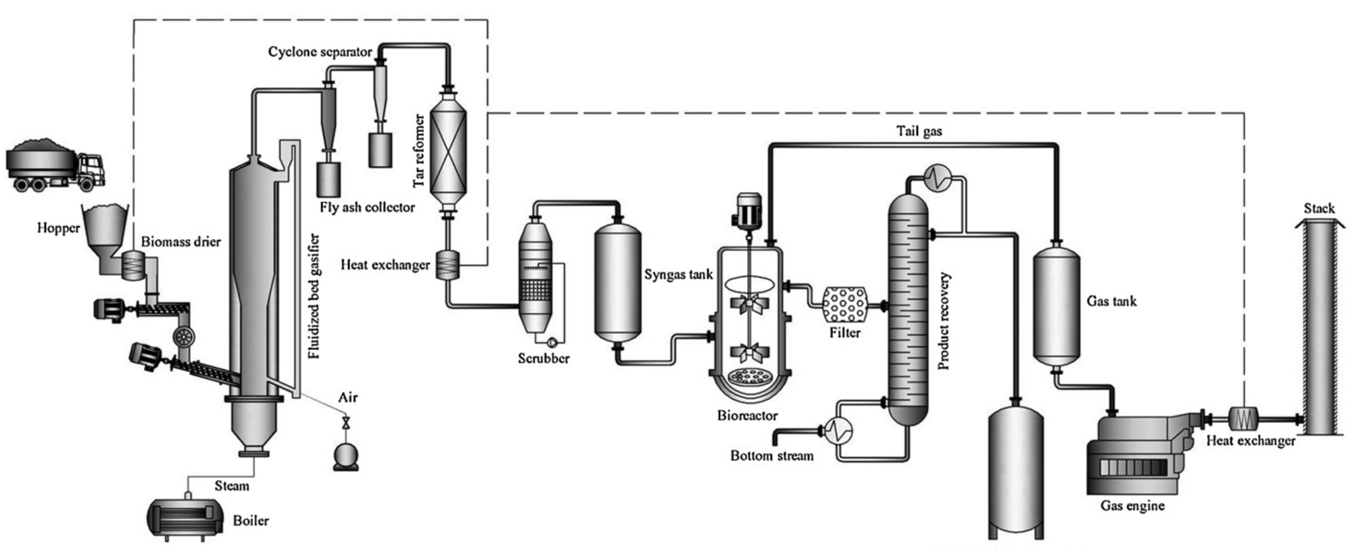


Fig. 2. Schematic representation of biomass gasification integrated with syngas fermentation to biofuels.

Table 1
Various microbes used to ferment gaseous substrates to biofuels.

	Fermentation product	Ref.
Rhodospirillum rubrum *Rhodobacter sphaeroides* *Methanobacterium thermoautotrophicum* *Methanosarcina barkeri* *Clostridium thermoaceticum* *Rhodopseudomonas gelatinosa* *Rhodopseudomonas capsulate* *Rhodopseudomonas palustris* *Rubrivivax gelatinosus* *Bacillus simithii* ERIH2 *Rhodopseudomonas palustris* P4 *Citrobacter amalonaticus Y19* *Rhodocyclus gelatinosus* *Carboxydothermus hydrogenoformans*	Hydrogen $CO + H_2O \rightarrow CO_2 + H_2$	[14,21,23–33]
Clostridium autoethanogenum *Clostridium ljungdahlii* *Moorella* sp. HUC22-1 *Acetobacterium kivui* *Peotostreotococcus productus* *Alkalibaculum bacchi*	Mixture of acetic acid and ethanol $6CO + 3H_2O \rightarrow CH_3CH_2OH + 4CO_2$ $2CO_2 + 6H_2 \rightarrow CH_3CH_2OH + 3H_2O$ $6CO + 6H_2 \rightarrow 2CH_3CH_2OH + 2CO_2$ $4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2$ $2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$	[14,22,34–38]
Eubacterium limosum *Clostridium carboxidivorans* *Butyribacterium methylotrophicum*	Mixture of ethanol, butanol, acetic acid and butyric acid $6CO + 3H_2O \rightarrow CH_3CH_2OH + 4CO_2$ $4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2$ $12CO + 5H_2O \rightarrow C_4H_9OH + 8CO_2$ $10CO + 4H_2O \rightarrow C_4H_9OH + CO_2$ $12H_2 + 4CO_2 \rightarrow C_4H_9OH + 7H_2O$	[14,39–46]
Peptostreptococcus productus *Eubacterium limosum* *Acetobacterium woodii* *Acetobacterium wieringae* *Clostridium thermoaceticum*	Acetic acid $2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$ $4CO_2 + H_2O \rightarrow CH_3COOH + 2CO_2$	[14,21,47–49]
Methanospirillum hungatii *Methanobacterium formicicum* *Methanobrevibacter smithu* *Methanosarcina barkeri* *Methanobacterium thermoautotrophicum*	Methane $4H_2 + CO_2 \rightarrow CH_4 + H_2O$ $4CO + 2H_2O \rightarrow CH_4 + 3CO_2$	[21,35]

2.2. Syngas fermentation

Syngas represents one of the most inexpensive and flexible substrate for biological fermentation process to produce a variety of renewable fuels and chemicals. Syngas can be generated from a plethora of organic materials including biomass thus offering the advantage of flexibility. It is also an inexpensive substrate, costing ≤ $6 per million Btu, with raw material cost of ≤$0.10/lb product [15].

Although there exist multiple pathways for the conversion of syngas, most transformations are performed through microbial or thermo-chemical processes [18]. Syngas fermentation is a potential microbial pathway in which anaerobic microorganisms are utilized to mediate the bio-catalytic conversion of syngas components to various useful biochemicals and biofuels [19]. These anaerobic microorganism can be classified as autotrophic or unicarbonotrophic. The autotrophs utilize the C_1 compounds in syngas including CO and/or CO_2 as the carbon source and H_2 as the energy source, whereas the unicarbonotrophs are able to use the C_1 compounds as their sole carbon as well as energy source [20]. A large variety of anaerobic microorganisms including photosynthetic, acetogenic, carboxydotrophic and methanogenic microbes are known to metabolize syngas to various end products [21]. Syngas fermentation can generate hydrogen, ethanol, butanol, acetic acid, butyric acid, methane, biopolymers and single cell protein (CSP) [22]. Table 1 summarizes various microorganism used to ferment syngas components to biofuels.

3. Advantages of biocatalysts

Although conversion of syngas to fuels using metal-based catalysts is a reliable technology that promotes instant reaction, however the catalytic process is restricted by some barriers. Drawbacks such as low catalyst selectivity, intensive operation cost due to the utilization of high temperatures and pressurized reactors, wide product distribution, requiring a specific ratio of gas components to yield a desired product and the possibility of catalyst poisoning by the trace amount of sulfur gases presented in the syngas are involved in catalytic conversion which contribute to the high cost of synthesis fuels [14,50]. The sulfur presented in syngas is usually in the form of hydrogen sulfide (H_2S) and carbonyl sulfide (COS), with smaller amounts of mercaptans or organic sulfur which are the main cause of acid rains. Processes such as Claus, liquid phase oxidation and absorption are typically implemented to reduce the sulfur content of syngas to less than 0.1 ppm which is safe for sensitive downstream catalysts [51].

Use of fermentation bacteria as biocatalyst has successfully offset several weaknesses of the traditional catalytic conversion process. First, biocatalysts operate at moderate temperature and pressure which result in substantial energy saving. Moreover, operation at ambient temperature avoids thermodynamic equilibrium relationship and causes the irreversibility of biological reactions, which consequently results in high conversion efficiencies [14,50,52]. Second, high reaction specificity is achieved in biological conversion in comparison to catalytic process due to the high enzymatic specificity. As a result of the high specificity, product yield is improved,

the recovery of product is simplified and less toxic by-products are evolved during the process [39,52]. Third, biocatalysts are less sensitive to the syngas components' ratio and do not require a fixed CO/H_2 ratio, whereas traditional catalysts need a specific ratio of gas components to yield a desired product. Forth, even toxic or recalcitrant organic feedstocks to enzymatic process can be fermented after gasification, as chemical difference between feedstocks is almost insignificant in the gasification process [39]. Finally, most biocatalysts can tolerate trace amount of contaminants such as sulfur and chlorine which is an advantage over metal-based catalysts. Even the growth of anaerobic bacteria can be stimulated in the presence of sulfur compounds, as sulfur acts as reducing agent which reduces the redox potential of the medium [17,53,54]. However, the syngas requires some clean-up before the fermentation process to maintain the maximum bacterial activity. Mechanical problems such as gas delivery line blockage, breaking and unstable gas flow may originate from char and tar accumulation in gas lines [19].

4. Biological water–gas shift reaction

Carbon monoxide in syngas reacts with water to produce H_2 and CO_2 through the water–gas shift reaction. The generated CO_2 is this reaction should be removed by absorption to yield pure hydrogen. This reaction may be alternatively used to improve the H_2 content of syngas for consequent applications. The water–gas shift reaction is slightly exothermic and a heterogeneous mixture of metals including chromium, zinc, iron, cobalt, copper, etc. is required to catalyze the reaction. Such process is restricted by disadvantages such as catalyst deactivation by sulfur, carbon deposition and high temperature [23].

In biological water–gas shift reaction hydrogenogenic organisms are utilized to produce H_2 by CO oxidation. The required energy to conduct the biological water–gas shift reaction is provided by electron transferring from CO to H_2O through the following coupled reactions:

$$CO + H_2O \rightarrow CO_2 + 2e^- + 2H^+ \tag{1}$$

$$H^+ + 2e^- \rightarrow H_2 \tag{2}$$

Carbon monoxide dehydrogenase (CODH) provides electrons and protons for the electron transport through the electrochemical half reaction (1) for CO and the hydrogenase enzyme supply energy for cell growth via catalyzing the hydrogen half reaction (2) [55,56]. The overall reaction obtained by sum of the two half reactions of (1) and (2) is:

$$CO + H_2O \rightarrow CO_2 + H_2 \quad \Delta G = -20.1\ \text{kJ/mol} \tag{3}$$

The biological water–gas shift reaction produces 4.46 kcal/mol CO, whereas the aerobic conversion would generate 61.1 kcal/mol CO through the following reaction [57]:

$$CO + O_2 \rightarrow CO_2 \tag{4}$$

Hence, a low level of energy is generated in anaerobic process which results in a slow cellular growth and prolonged time to reach the steady–steady. It has been reported *Rubrivivax gelatinosus* is able to produce 1.4 g of cells per 1 mol of CO under anaerobic condition, whereas its aerobic growth on acetate, yields in production of 2.0 g of cell per 1 mol of acetate [57]. On the contrary, since much less energy is produced in anaerobic reaction (2–3 ATP) in comparison to aerobic respiration (26–38 ATP), a cell has to consume almost ten times the amount of substrate to obtain the same level of energy. Thus, a high turnover of substrate is achieved in anaerobic process which could be an advantage for biotechnology [58]. It is also worth noting that, although aerobic reaction provides more energy for the organism and high cell population is achieved, no H_2 would evolve during the aerobic reaction.

Yeol Jung et al. [26] isolated a bacterium, *Citrobacter* sp. Y19, from an anaerobic wastewater sludge digester to catalyze the water–gas shift reaction. It was observed that the bacterial growth was much faster under aerobic condition in comparison to anaerobic condition. However, neither CO consumption nor H_2 production was observed under aerobic condition. Thus, it was planned to cultivate the bacteria in two steps; growing the bacteria in aerobic condition followed by anaerobic process for H_2 production. The anaerobically grown bacteria were able to produce 33 mmol H_2/g cell and reached conversion efficiency close to 1.0 for a prolonged fermentation time of 250 h, while 1.0 atm CO was repeatedly replenished in bottles. Similar behavior was observed with the bacterium *Rhodopseudomonas palustris* P4 [25]. Although the aerobic cultivation of the bacteria resulted in a dense culture, but no evolution of H_2 occurred in aerobic condition. Thus, the bacteria were first grown in aerobic–chemoheterotrophic condition and then transferred to an anaerobic bioreactor for H_2 production. In this experiment, a cell density of 10 g/l and maximum H_2 productivity of 41 mmol/g h was achieved in continuous fermentation. While, anaerobic–phototrophic growth of the bacteria followed by anaerobic cultivation resulted in a cell density of 1 g/l and H_2 productivity of 10 mmol/g h. In the case of these bacteria, although CO is not required for oxidation or assimilation of the bacterial growth, however its presence is necessary to induce the H_2 production activity. It is plausible that the synthesis of the CO-dependent H_2 producing enzymes evolves during the aerobic growth phase and become activated during the subsequent anaerobic cultivation process [59].

Biological water–gas shift reaction which utilizes hydrogenogenic organisms to form H_2 by oxidizing CO is highly depended on the performance of catalyst and various strains have been isolated to conduct this reaction [60]. A list of the various hydrogenogenic organisms utilized to generate H_2 and the summary of the implemented conditions for CO fermentation is tabulated in Table 2.

Generally, hydrogen producing bacteria are categorized as heterotrophic anaerobes, heterotrophic facultative anaerobes, heterotrophic strict anaerobes and photosynthetic bacteria [24]. Photosynthetic bacteria are versatile to catalyze the biological water–gas shift reaction. Production of H_2 by these bacteria is highly depended on the presence and activity of the hydrogen producing enzymes. Photosynthetic bacteria have four terminal enzymes of nitrogenase, uptake hydrogenase, fermentative hydrogenase, and CO-linked hydrogenase to mediate the H_2 metabolism [69]. These enzymes are involved in H_2 evolution under various growth conditions. Several photosynthetic bacteria, able to convert CO to H_2 and CO_2 are *Rhodobacter sphaeroides*, *Rhodopseudomonas gelatinosa*, *Rhodopseudomonas palustris*, *Rhodocyclus gelatinosus*, *Rubrivivax gelatinosus*, *Rhodospirillum rubrum*, *Methanosarcina barkeri, Citrobacter* sp. Y19, *Methanobacterium thermoautotrophicum* and *Clostridium thermoaceticum* [26,29]. Among the photosynthetic bacteria, the purple non-sulfur bacteria such as *Rh. rubrum* and *Ru. gelatinosus* have attracted considerable attention due to their high CO uptake rate and high CO conversion close to the theoretical value of 1.0 [25,70]. However, it should be noted that various strains of these bacteria may act differently [23].

Photosynthesis anoxic bacteria are able to utilize light energy and organic compounds to generate electrons. These bacteria contain some photo-reactive pigments through which light energy is absorbed and converted to chemical potential. The generated chemical potential is then involved in microbial metabolism. Thus, in these bacteria cell pigments play an important role in the light absorption capability of the organism. The light intensity affects the cell growth and product evolution. Although the high light intensities promote the bacterial cell growth due to the assimilation of

Table 2
Production of hydrogen using various hydrogenogenic bacteria.

Organism	Substrate (v/v, %)	Culture mode	Time	Temp. (°C)	pH	Cell density (g/l)	Conversion efficiency	Product	Ref.
Rhodospirillum rubrum	$CO/H_2/CO_2/Ar$ (55:20:10:15)	Batch	120 h	30	7.5	0.27	X_{CO}: 88%	0.86 mmol H_2/mmol CO 0.063 mmol H_2/g h	[61]
Rhodospirillum rubrum	$CO/H_2/CO_2/Ar$ (55:20:10:15)	Batch	120 h	30	6.9	na	X_{CO}: 86%	16.9 mmol H_2/l	[62]
Rhodospirillum rubrum	$CO/H_2/CO_2/Ar$ (55:20:10:15)	CSTR	600 h	30	6.3	3.4	X_{CO}: 80%	0.82 mmol H_2/mmol CO 16 mmol/g h	[24]
Rhodospirillum rubrum	$CO/H_2/CO_2/Ar$ (55:20:10:15)	CSTR	7 days	na	6.3	na	X_{CO}: 60%	9.6 mmol H_2/h	[63]
Bacillus simithii ERIH2	$CO/H_2/CO_2/CH_4$ (64.34:20.8:10.8:4.06)	CSTR	na	50	6.4–6.9	0.68	X_{CO}: 80%	H_2 yield 95%	[23]
Rhodopseudomonas palustris P4	N_2/CO (90:10)	Aerobic–chemoheterotrophic cell growth/anaerobic CSTR	400 h	30	7.0	10	$X_{CO} > 60$	41 mmol/g h	[64]
Citrobacter sp. Y19	N_2/CO (92:8)	Aerobic–chemoheterotrophic cell growth/anaerobic CSTR	68 h	30	7.0	~1.5	na	20 mmol H_2/g h	[59]
Rubrivivax gelatinosus CBS	$CO/CH_4/N_2$ (20:5:75)	Trickle-bed rector	na	30	7.0	na	Specific CO uptake rate 0.73 mmol/min/g	0.8 mmol H_2/g min	[65]
Rubrivivax gelatinosus CBS	CO/N_2 (10:90)	Hollow fiber immobilized cell	8 months	25–26	6.8	0.65	na	125 ml H_2/g h	[66,67]
Rubrivivax gelatinosus CBS	CO/N_2 (20:80)	Bubble train bioreactor	10 days	25–26	na	na	na	140 ml H_2/g h	[66,67]
Rhodospirillum rubrum	$CO/H_2/CO_2/N_2$ (55:20:10:15)	CSTR	120 h	30	7.0	na	X_{CO}: 58%	0.88 mol H_2/mol CO 0.01 mol H_2/g h	[68]

na: not available.

organic sources in the presence of light, however, excess of the light intensity can decline the metabolic activity of the bacteria. In such cases, the dense color of the pigments prevents the full penetration of light into the cells [71]. Therefore, the optimum light intensity is an important parameter that needs to be carefully determined. In this aspect, Najafpour and Younesi [71] investigated the effect of light intensity (500, 1000 and 1500 Lux.) on microbial cell metabolism and H_2 evolution in a batch culture of *R. rubrum*. Also the concentration of acetate (1, 1.5 and 2 g/l) as a carbon source in the presence of syngas was varied in the media. The result of their survey explored the major role of acetate concentration in cell propagation as well as the influence of light intensity on cell concentration. It was realized that, the both energy sources of light and organic substrate were required to provide electron for the bacteria to produce H_2. However, while the light intensity or acetate concentration exceeded the limit, the microbial growth was retarded. In their experiments, maximum CO conversion of 98% and yield ($Y_{H2/CO}$) of 86% was achieved at the acetate concentration of 1.5 g/l and light intensity of 1000 Lux. Jung et al. [72] also experimented the effect of light on growth and product evolution of the bacterium, *R. palustris P4*, with CO as the sole carbon source. From the typical features of *Rhodopseudomonas* species, it was known that this facultative anaerobe was able to grow either in photoautotrophic or chemo-heterotrophic manner. Nevertheless, formation of purple pigment was under the photoautotrophic conditions only. This was further confirmed when no growth was observed in the absence of light, indicating that this bacterium requires light and cannot utilize CO as energy source. When light was supplied, cell growth and CO consumption was fast, however H_2 was not evolved, unless the light was removed. It was realized that the elimination of light was required to induce H_2 production along with CO utilization through the water–gas shift reaction, as some ATP is synthesized in this reaction that P4 could obtain maintenance energy from it.

5. Acetogenic bacteria

Three class of bacteria contribute to acetate production in anaerobic process; fermentative bacteria, syntrophic acetogens and homoacetogens. Fermentative bacteria and acetogens are able to produce acetate, H_2, CO_2 and other products, whereas homoacetogens utilize H_2 and CO_2 to produce acetate. [34,73,74]. Homoacetogenic bacteria which are also called acetogens, can grow on H_2 plus CO_2 as carbon and energy source, and the reduction of CO_2 to acetate is coupled with net ATP synthesis [75].

Acetogens are obligate anaerobic bacteria able to grow chemolithotrophically on various substrates including C_1 compounds such as carbon monoxide, carbon dioxide plus hydrogen, formate and methanol and ferment them to volatile fatty acids and alcohols such as acetate, butyrate and ethanol, but mainly acetate, under ambient temperature and pressure [50,54,76,77]. Some of the acetogenic bacteria are *Clostridium thermoaceticum*, *Clostridium formicoaceticum*, *Eubacterium limosum*, *Acetobacterium woodii*, *Acetogenium kivuii*, *Clostridium thermoautotrophicum*, *Clostridium ljungdahlii* and *Butyribacterium methylotrophicum* [39,76,78]. A list of the various acetogenic bacteria for production of acetate, ethanol, butyrate and butanol and the summary of the implemented conditions for syngas fermentation is illustrated in Table 3.

5.1. Pathway of acetogens

The Wood–Ljungdahl pathway for acetogens was first elucidated by Wood and Ljungdahl. This pathway governs the conversion of CO and H_2 to acetyl-CoA and products such as acetate. Since this pathway involves acetyl-CoA as an intermediate and CODH as the key enzyme, thus it is often called "acetyl-CoA pathway" or "carbon monoxide dehydrogenase pathway" [75]. The acetogenic bacteria utilize the acetyl-CoA pathway as the predominant mechanism to synthesize acetyl-CoA from CO_2 [14]. Generally, organisms which utilize CO have also the ability of utilizing CO_2 and CO_2/H_2 through the same set of enzymes and transformation mechanism encompassed by the acetyl-CoA pathway. In fact, the capability of the microorganisms to convert CO_2 to acetate through a H_2-dependent route was found long before demonstrating that the same organisms could use CO and that the same pathways were involved [15].

The acetyl-CoA pathway is a terminal electron accepting process which conserves energy and is used for the CO_2 assimilation into biomass and cell components, especially in autotrophic organisms [89]. This pathway is an irreversible, non-cyclic path that takes place under strictly anaerobic condition and governs acetogenic bacterial fermentation, [85,90]. The net ATP formation to provide energy for the growth of cells is zero for this pathway [55]. The pathway for acetyl-CoA synthesis consists of two methyl and carbonyl branches, through which CO_2 is reduced to methyl and carbonyl level via several enzyme dependent reactions. This pathway is summarized in the scheme in Fig. 3 [14,15,75,91–93]

In methyl branch of the acetyl-CoA pathway, CO_2 is reduced to formate ($HCOO^-$) at the first step, as shown in the following equation [94]:

$$CO_2 + NADPH \rightarrow HCOO- + NADP^+ \tag{5}$$

This reaction is reversibly catalyzed by the formate dehydrogenase (FDH) enzyme, whose function is to reduce CO_2 to formate [14,94]. The generated formate is then the precursor for the methyl group synthesis of the acetyl-CoA pathway. The formate is activated by tetrahydrofolate (H_4folate) to form 10-formyl-H_4folate catalyzed by 10-formyl-H_4folate synthetase through the following reaction [17,94]:

$$HCOOH + H_4folate + ATP \rightarrow HCO\text{–}H_4folate + ADP + P_i \tag{6}$$

The enzyme cyclohydrolase catalyze the further conversion of this intermediate to yield 5,10-methenyl-H_4folate cyclohydrogenase [17,94]:

$$HCO\text{–}H_4folate + H^+ \rightarrow CH\text{–}H_4folate^+ + H_2O \tag{7}$$

In the next NADPH-dependent reduction, the 5,10-methenyl-H_4folate is converted to 5,10-methylene-H_4folate by the methylene-H_4folate dehydrogenase enzyme [17,94]:

$$CH\text{–}H_4folate^+ + NADPH \rightarrow CH_2\text{–}H_4folate + NADP^+ \tag{8}$$

Then, the enzyme methylene-H_4folate reductase reduces this intermediate to (6S)-5-methyl-H_4folate [94]:

$$CH_2\text{–}H_4folate + ferredoxin_{red} \rightarrow CH_3\text{–}H_4folate + ferredoxin_{ox} \tag{9}$$

At the final stage of the methyl synthesis, CH_3–H_4folate is transferred to the cobalt centre of the corrinoid/iron–sulfur protein. The corrinoid protein must be reduced to accept a methyl group from 5-methyl-H_4folate. This reduction is carried out by reduced ferredoxin which may be generated using pyruvate and pyruvate–ferredoxin oxidoreductase or CO and CODH as follows [94]:

$$[Co^{+3}\text{-E}] + 2ferredoxin_{red} \rightarrow [Co^+\text{-E}] + 2ferredoxin_{ox} \tag{10}$$

Then, the reduced corrinoid protein is methylated by transmethylase through the following reaction [94]:

$$[Co^+\text{-E}] + CH_3\text{–}H_4folate \rightarrow [CH_3\text{–Co-E}] + H_4folate \tag{11}$$

In the carbonyl branch of the acetyl-CoA pathway, a carbonyl group is formed which is then merged with the methyl group, synthesized in the methyl branch, to form acetyl-CoA. The enzyme

Table 3
Production of biofuel using various acetogenic bacteria.

Organism	Gas substrate (v/v, %)	Culture mode	Fermentation time	Temperature (°C)	pH	Cell density (g/l)	Conversion efficiency	Product	Ref.
Moorella sp. HUC22-1	H_2/CO_2 (80:20)	Batch	220 h	55	6.2	0.92	na	0.20 mmol acetate/mmol H_2 0.42 mmol acetate/mmolCO_2	[34]
Moorella sp. HUC22-1	H_2/CO_2 (80:20)	Cell recycled repeated batch	420 h	22	6.2	1.51	na	7.2 mmol acetate/g h	[79]
Clostridium aceticum	$CO/H_2/Ar$ (78:4:18)	Batch	72 h	30	8.5	0.80	X_{CO}: 100%	2.27 g acetate/l	[76]
Clostridium ljungdahlii	$CO/H_2/CO_2/Ar$ (55:20:10: 15)	Cell recycled CSTR	560 h	36	4.5	4	X_{CO}: 90% X_{H2}: 70%	48 g ethanol/l 3 g acetate/l 21 mol ethanol/mol acetate	[47]
Acetogenium kivui	$CO/H_2/CO_2/N_2$ (13:14:5:68)	CSTR	na	na	6.6	5.14	X_{CO}: 69% X_{H2}: 37%	19.16 g acetate/l 1.13 g/l h	[22]
Clostridium ljungdahlii ERI2	$CO/H_2/CO_2/N_2$ (13:14:5:68)	ICR	na	na	4.9	0.18	X_{CO}: 100% X_{H2}: 78.6%	5.604 g acetate/l 2.743 g ethanol/l	[22]
Clostridium ljungdahlii ERI2	$CO/H_2/CO_2/N_2$ (14:17:4:65)	TBR	na	37	5.05	13.6	X_{CO}: 57% X_{H2}: 58%	6.4 g acid acetic/l	[22]
Clostridium ljungdahlii isolate O-52	$CO/H_2/CH_4$ (45:50:5)	CSTR	na	37	5.05	na	X_{CO}: 85% X_{H2}: 50%	21 g ethanol/l 3 g acetic acid/l	[22]
Clostridium ljungdahlii	$CO/H_2/CO_2/Ar$ (55.25:18.11:10.61: 15.78)	Two-stage CSTR	na	37	5 in the first, 4–4.5 in second	na	na	1 g ethanol /l 3 g acetate/l 1 mol ethanol/acetate 4 mol ethanol/acetate	[80]
Clostridium ljungdahlii	$CO/H_2/CO_2/N_2$ (20:10:20:50)	Liquid batch, continuous gas	44 h	37	6.8	0.562	na	0.29 mmol ethanol/g h 0.127 mmol ethanol/mmol acetate	[81]
Clostridium autoethanogenum	$CO/H_2/CO_2/N_2$ (20:10:20:50)	Liquid batch, continuous gas	72 h	37	na	0.150	na	1.45 mM ethanol 23.3 mM acetate	[81]
Clostridium autoethanogenum	CO/CO_2 (95:5)	Batch	60 h	37	4.74	na	*Continued*	259 mg ethanol/l	[82]
Clostridium autoethanogenum	60% CO, balance is not defined	Batch	na	37	6	na	X_{CO}: 97%	7.71 mmol ethanol/l 7.96 mmol acetate/l	[21]
Butyribacterium methylotrophicum	100% CO	Batch with continuous CO sparge	na	37	pH shift from 6.8 to 6	0.31	na	5.95 g butyrate/l 3.88 g acetate/l	[83]
Butyribacterium methylotrophicum	100% CO	CSTR	na	37	6	0.286	na	0.536 g butyrate/l	[41]

Table 3 (*Continued*)

Organism	Gas substrate (v/v, %)	Culture mode	Fermentation time	Temperature (°C)	pH	Cell density (g/l)	Conversion efficiency	Product	Ref.
Clostridium carboxidivorans P7^T	$CO/CO_2/N_2$ (25:15:60)	Bubble column bioreactor	30 days	37	5.8–5.9	na	na	0.689 g acetate/l 0.56 g ethanol/l Apparent yields (mole C in products per mole CO consumed) ethanol: 0.15 Butanol: 0.075 Acetic acid: 0.025	[42]
Clostridium ljungdahlii	$CO/H_2/CO_2/Ar$ (55:20:10:15)	Batch	120 h	37	na	1.2	X_{CO}: 100%	1.3 g acetate/l 0.6 g ethanol/l	[84]
Clostridium strain P11	$CO/H_2/CO_2/N_2$ (20:5:15:60)	Batch	15 days	37	5.9	na	na	2.67 g ethanol/l	[85]
Eubacterium limosum	CO/CO_2 (80:20)	Batch	54 h	37	6.8	0.434		0.758 mmol acetate 0.001 mmol butyrate	[86]
Eubacterium limosum	100% CO	Gas lift reactor	2 days	37	6.8	0.750		0.172 mol acetate/mol CO 0.014 mol butyrate/mol CO 0.18 mol acetate/mol CO	[87]
Clostridium carboxidivorans	$CO/CO_2/N_2$ (25:15:60)	Bubble column bioreactor	17 days	37	5.2	0.35	X_{CO}: 60%	38 mmol ethanol/l 0.33 mol ethanol/mol CO (based on carbon content) 0.033 mol butanol/mol CO 0.04 mol acetate/mol CO	[45]
Clostridium strain P11 (*Clostridium ragsdalei*)	$CO/H_2/CO_2/N_2$ (20:5:15:60)	CSTR	59 days	37	~5	1.13	na	25.26 g ethanol/l (59 days) 9.25 g 2-propanol/l (24 days) 4.82 g acetate/l (24 days) 0.47 g butanol/l (13 days)	[52]
Clostridium ljungdahlii	H_2/CO_2 (75:25) or CO/CO_2 (80: 20)	Batch	28 h	37	4.5	~0.1 (H_2/CO_2) ~0.25 (CO/CO_2)	na	0.297 mol ethanol/mol acetate (H_2/CO_2) 0.658 mol ethanol/mol acetate (CO/CO_2)	[55]
Butyribacterium methylotrophicum	CO/CO_2 (70:30)	Batch	144 h	37	6.9	~0.35	X_{CO}: 100%	~1.3 g acetic acid/l ~0.4 g butyric acid/l ~0.08 g ethanol/l	[46]
Clostridium ragsdalei	$CO/H_2/CO_2/N_2$ (20:5:15:60)	Batch	15 days	32	6	0.95	na	1.89 g ethanol/l 1.45 g acetate/l	[88]

na: not available.

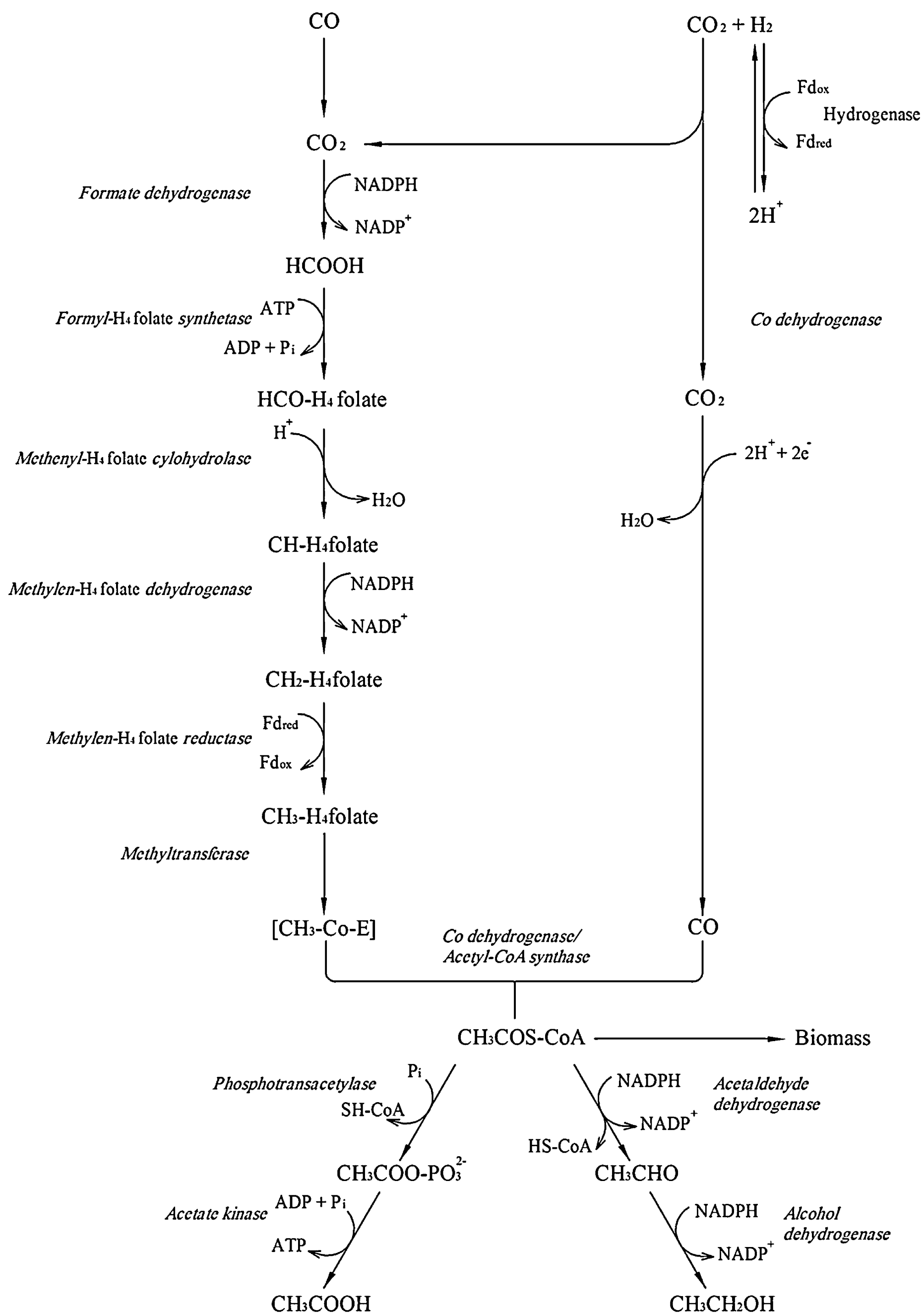


Fig. 3. The acetyl-CoA pathway for acetogenic microbes.

CODH plays an important role in the carbonyl branch of the pathway. It reduces CO_2 to CO as shown in the following equation [14]:

$$CO_2 + 2H^+ + 2e^- \rightarrow CO + H_2O \quad (12)$$

The key enzyme Ni–CODH is classified as: (1) monofunctional CODH, which catalyzes the oxidation of CO to CO_2, which could be consequently reduced to formate and then methyl group in acetyl-CoA pathway and (2) bifunctional CODH, which reduces CO_2 to CO as the carbonyl group in acetyl-CoA synthesis and also mediates the evolution of acetyl-CoA alongside the acetyl-CoA synthase (ACS) [17]. At the final stage of acetyl-CoA synthesis, CO (carbonyl moiety) condenses with the Co-methyl group (methyl moiety) of the methylated corrinoid protein and coenzyme A to yield acetyl-CoA. This reaction is catalyzed in the presences of CODH/ACS as follows [17,94]:

$$[CH_3\text{–Co-E}] + CO + \text{HS-CoA} \rightarrow CH_3COS\text{–CoA} + [\text{Co-E}] \quad (13)$$

The generated acetyl-CoA is an ideal precursor for the synthesis of the cell materials including amino acids, nucleotides, lipids and carbohydrates [94]. Based on anabolic or catabolic pathway, acetyl-CoA can be used as a source of cellular carbon or cellular energy. In the anabolic pathway, acetyl-CoA is carboxylated to pyruvate in the presence of pyruvate synthase. Then, the pyruvate is converted to phosphoenolpyruvate, which is considered as an intermediate in the evolution of cell materials. In contrast, in catabolic pathway the acetyl-CoA undergoes some reaction to generate ATP and acetate.

The conversion of acetyl-CoA to acetate is carried out via formation of acetyl-phosphate ($CH_3COO–P_3{}^{2-}$) as the intermediate [14,75]:

$$CH_3COS–CoA + Pi \rightarrow CH_3COO–P_3{}^{2-} + HS\text{-}CoA \quad (14)$$

This reaction is catalyzed by phosphotransacetylase. In the next reaction, acetyl-phosphate is transformed to acetate, while a molecule of ADP is phosphorylated to ATP in the presence of acetate kinase [75]:

$$CH_3COO–P_3{}^{2-} + ADP \rightarrow CH_3COOH + ATP \quad (15)$$

This phase of metabolism which results in acetate production is often known as the acidogenic phase and is preferred by the bacteria to alcohol forming pathway. Conversion of acetyl-CoA to acetate and ATP is carried out in growth phase, whereas evolution of ethanol and NADP is performed during the non-growth phase. Thus in the solventogenic phase that ethanol is produced from acetyl-coA, the growth is slow and no ATP is evolved in this phase. In this fermentation phase, the reducing potential in the form of NADH is utilized by the organism to form acetaldehyde (CH_3CHO) in the presence of acetaldehyde dehydrogenase [14]:

$$CH_3COS–CoA + NADH + H^+ \rightarrow CH_3CHO + NAD^+ + HS\text{-}CoA \quad (16)$$

$$CH_3CHO + NADH + H^+ \rightarrow CH_3CH_2OH + NAD^+ \quad (17)$$

The generated acetaldehyde is converted to ethanol by the enzyme alcohol dehydrogenase.

6. Syngas fermentation conditions

Various operation parameters involved in fermentation process include: nutrient constituents and concentrations, temperature, medium pH, pressure, inoculums level, liquid flow rate, gas flow rate, agitation speed while using stirred tank bioreactors, maximum gas concentration to avoid substrate inhibition and maximum product concentration in the media to avoid product inhibition [22]. The optimum values of these parameters should be carefully implemented and controlled during the fermentation process to improve the cell growth and product yield.

6.1. Effect of media composition

Bacteria as one of the living organism require a source of energy to survive and grow. All bacteria need elements such as carbon, nitrogen, sulfur and phosphorous for synthesis of cell materials and products [50]. In syngas fermentation, syngas constituents are utilized by bacteria as the carbon and energy source. Besides, various minerals and vitamins are required to maintain a high metabolic activity [17].

There is a hypothesis that solvent production in favor of acid production by homoacetogentic bacteria is promoted in non-growth condition which may be achieved by nutrient limitation (e.g. carbon-, nitrogen- and phosphate-limited medium), reducing agent addition, pH shift, hydrogen addition and alternate medium constituents [17,35,95]. Study of the acetyl-CoA pathway revealed that acetate production is balanced in ATP, whereas ethanol production gives rise to ATP consumption which would not support the bacterial growth. Hence, to enhance ethanol production over acetate, factors which would not support the growth of bacteria should be considered and the non-growth condition would be implemented after a desired cell concentration in the culture is achieved [35,45,95]. Such case was studied by Phillips et al. [47] who optimized the fermentation medium of *C. ljungdahlii* to improve ethanol production over acetate. They reduced the B-vitamin concentration and eliminated yeast extract from media. As a result, the cell growth potential slightly diminished, however ethanol was predominantly produced over acetate. Maximum ethanol concentration of 48 and 23 g/l was achieved in CSTR studies with and without cell recycling, respectively. Also, maximum ethanol to acetate ratio of 21 mol/mol was obtained with cell recycling. Gaddy and Clausen [80] experimented the effect of yeast extract concentration in the medium on product distribution during the batch fermentation of *C. ljungdahlii*. At low yeast extract levels (0.005, 0.01 and 0.05%), a molar ethanol to acetate ratio of 0.11 was achieved, whereas this ratio was 0.05 at higher levels of yeast extract (0.1 and 0.2%). Such observation further confirmed the promoted ethanol production in non-growth condition created by the reduced level of yeast extract in the medium. In another experiment carried out by Klasson et al. [35], the importance of media composition during the batch fermentation of *C. ljungdahlii* was considered. There was a premise that forcing the bacterial culture to grow at a reduced rate would induce the sporulation and improve ethanol production. During the fermentation, syngas was the primary carbon source, but yeast extract was replaced with cellobiose, rhamnose, galactose and starch, each in a separate experiment. Use of these nutrients instead of yeast extract brought about sporulation and improved the cell and ethanol concentrations as well as the product ratios. The highest cell concentration of 170 mg/l, ethanol concentration of 0.56 mmol and ethanol to acetate ratio of 0.45 was achieved with cellobiose.

Nutrient limitations can cause some limitations in cell metabolism such as its maintenance, intracellular enzyme production and cofactor formation. Such condition induces the non-growing or resting cell state which shifts the metabolic pathway from the acidogenic to solventogenic phase. However, if the culture suffers from significant loss in cell viability and the metabolic activity level becomes too low, the cells may not be able to generate the correct enzymes and cofactors such as acetaldehyde dehydrogenase, acetate kinase and NADH or utilize the electron carriers to uptake the carbon substrate or metabolic intermediates [95]. In an effort to trigger the non-growing state to promote ethanol production which is known as a non-growth associated metabolite in autotrophic *Clostridia* sp., Cotter et al. [95] examined the effect of nitrogen-limited media on ethanol and acetate production capabilities in *C. autoethanogenum* and *C. ljungdahlii* cultures. Ammonium chloride, proteose peptone, yeast extract and beef extract were utilized as various sources of nitrogen in the media. In their experiments, little to no ethanol production was observed in non-growing *C. ljungdahlii* culture which was probably due to the low metabolic function enabling the substrate conversion, as indicated by the minimum level of substrate consumption. In contrast, improved ethanol production capability was observed with *C. autoethanogenum* which showed the bacteria were extremely sensitive to nitrogen limitations. Maness and Weaver [96] experienced the condition for synthesis of poly-3-hydroxyalkanoate (PHA), from a gaseous substrate in a nutrient imbalance culture of *Rhodobacter* sp. *CBS*. PHA is a high molecular weight thermoplastic which is formed as a portion of cell mass that can be solvent extracted. It was found that under unbalanced culture condition while the cellular growth was limited by deficiency of nitrogen, basal salt ($MgSO_4$, $CaCl_2$, and $FeSO_4$) and essential vitamins, up to 28% of the synthesized cell mass was granules of PHA. It was also known that the yield of PHA was depended to the type and amount of organic acid available in the medium. Thus, by addition of exogenous acetate to the culture growing on CO and H_2, the PHA content of cell dry mass improved to 79%.

Some efforts were made to reduce the cost of fermentation media which is considered as an important criterion for large scale biofuel production plants. To this end, Kundiyana et al. [85], attempted to utilize cotton seed extract (CSE), without addition of any medium component, as the fermentation medium for *Clostridium* strain P11 to produce ethanol. It was declared that CSE contains some minerals and vitamins which are very similar to the standard

fermentation medium for *Clostridium* strain P11, also containing eighteen amino acids. They reported successful production of 2.66 g/l ethanol in the batch medium containing 0.5 g/l of CSE after 15 days of operation, while a low concentration of acetic acid was detected in the medium. It was suggested that media components including vitamins and mineral salts used in standard fermentation medium of *Clostridium* strain P11 could be replaced with CSE to reduce the process cost.

6.2. Effect of organic source

Carbon monoxide as one of the major components of syngas or any other flue gas is a suitable renewable feedstock for microbial fermentation in spite of its high toxicity. CO can be metabolized by various bacteria such as aerobic carboxydotrophs and anaerobic acetogens, phototrophs, sulfate-reducers and methanogens [97]. Nevertheless, the anaerobic fermentation of CO would be preferable as the available electrons are used to generate relatively reduced biochemicals rather than being lost to oxygen [83]. Anaerobic microorganisms able to grow either chemolithotrophically on substrates such as CO and H_2/CO_2, which are considered as the main syngas constituents, or chemoorganotrophically with carbon sources such as fructose, acetate, malate, glutamate, furmarate, succinate and pyruvate, are suitable candidates for this purpose. They utilize substrates such as CO or CO_2 during the fermentation process to provide energy for bacterial growth and maintenance and also metabolize the production of by-products such as organic acid, alcohols and hydrogen [84].

Cotter et al. [81] compared the growth of *C. ljungdahlii* on syngas (50%N_2, 20% CO, 20% CO_2 and 10% H_2) and fructose. The bacterial growth on sugar-based carbon resulted in a dense culture (1 g/l) compared to syngas (0.562 g/l). Also, the ethanol concentration achieved with fructose (13 mM) was considerably higher than that of syngas (3.8 mM). Such difference in growth performance was probably due to the diffusion limitations at the gas–liquid interface and/or efficiency of the uptake and transport mechanism of the gaseous substrate. They also observed that syngas fermentation inoculated with cells pre-cultured on fructose resulted in a higher cell density (0.850 g/l) in comparison to the cells pre-culture on syngas (0.562 g/l). It was inferred that such difference in culture performance was likely attributed to a greater availability of intracellular cofactors, enzymes and maintenance energy in cells adapted to sugar substrate. In another experiment [98], they investigated the effect of cell pre-adaptation to various carbon sources (fructose, fructose–syngas and syngas) on cell growth and product formation while fermenting the syngas. It was found that the highest cell growth and ethanol production was obtained with cells pre-adapted to fructose with almost 2.5 and 1.5 times more ethanol production than that of syngas and syngas–fructose pre-adapted cells, respectively. Pre-adaptation of cells with fructose probably provided higher level of energy equivalents (ATP/GTP) and reducing power (NADH/NADPH) to utilize syngas as the carbon source.

Gaddy and Chen [23] isolated a hydrogen producing bacterium, *Bacillus simithii* ERIH2, which was able to consume CO rapidly to yield H_2. The anaerobic strain ERIH2 was very effective in catalyzing the biological water–gas shift reaction and showed a CO uptake rate of nearly 20 times higher than that of *R. rubrum* in batch culture, without the need for light. However, the bacterium suffered from poor growth rate. Thus, some growth supplements were implemented in bacterial culture to stimulate the cell growth. The medium contained vitamins, salts, minerals, trypticase, yeast extract and glucose as the cheap carbon source. The result of this investigation showed that during the first 8 h of incubation, the cell concentration considerably increased, however the bacterial growth was mainly on trypticase and yeast extract and glucose consumption was slow during this period. After the initial 8 h, glucose was consumed at a higher rate. Therefore, it was deduced that the glucose transfer across the bacterial cell membrane was the growth limiting step. Since the bacterium ERIH2, did not utilize CO as a carbon source for growth, an adequate concentration of glucose was required to provide a high cell density. They concluded that use of a rich media containing 25 g/l glucose, 5 g/l trypticase and 20 g/l yeast extract resulted in high cell concentration of 5 g/l. In another investigation conducted by Najafpour et al. [61] the effect of various carbon sources on biomass and H_2 production yield through biological water–gas shift reaction was studied. The bacteria, *Rhodospirillum rubrum*, were grown on three carbohydrates of glucose, fructose and sucrose and three organic acids of formate, acetate and malate, while the gas phase contained 55% CO, 20% H_2, 10% CO_2 and 15% Ar. The obtained results revealed that the highest yield of biomass ($Y_{\text{g cell/g substrate}} = 0.47$) was obtained with fructose, whereas acetate was the most favorable substrate to achieve the highest H_2 production yield of 0.86 mmol H_2/mmol CO. They also concluded that malate was a suitable substrate for the cell growth, but not for H_2 production. They suggested acetate as the favorable carbon source for H_2 production due to the ability of acetate to be converted to acetyl-CoA to initiate the tricarboxylic acid (TCA) cycle, which is known as the H_2 production pathway in purple nonsulfur bacteria such as *R. rubrum* [99]. In an effort to determine the influence of media composition on ethanol and acetate production using *C. ljungdahlii*, Gaddy and Clausen [80] utilized a medium containing yeast extract, mineral salts and vitamins without any carbon source like CO or syngas. In this experiment, no alcohol was evolved at the medium pH of 5, whereas significant amount of organic acids was formed, probably due to the utilization of yeast extract as a carbon source by the bacteria.

6.3. Effect of medium pH

Fermentation pH is one of the influential parameters in regulating the substrate metabolism and altering the physiological parameters including the internal pH, membrane potential and proton-motive force. As a result, the medium pH affects the release of metabolic by-products, product selectivity and composition [100]. There is a narrow range for every organism, in which the cells are metabolically active. Any upset in pH can lead to damage or death of the cells and consequently results in loss of biological activity. Lowering the medium pH diminishes the cell growth and affects the overall productivity of the process, because of the reduced flow of carbon and electron from the substrate toward the cell mass. However, in the case of acetogenic bacteria, this is an advantage because the product spectrum shifts from acidogenic to solventogenic phase, which is favored for production of highly reduced products such as ethanol [17]. In such cases, the generated acetic acid which is a weak organic acid permeates through the cell membrane, as it is a lipophilic acid in the undissociated form. Acetic acid conduct H^+ ions while diffusing through the cell membrane and thus reducing the intracellular pH. At the low internal pH values, external pH plays an important role in counteracting this situation by producing solvents [85].

In an effort to produce butyrate from 100% CO by *Butyribacterium methylotrophicum*, Worden et al. [83] studied the medium pH as an influential parameter to induce butyrate production. Batch fermentation with continuous CO sparging initiated at medium pH of 6.8, where acetate was formed in the medium but no evolution of butyrate was observed. Thus, at the onset of stationary phase with a cell concentration of 0.31 g/l, the pH was shifted from 6.8 to 6.0. As a result, a considerable increase in butyrate production was observed in favor of acetate and a final butyrate concentration of 6 g/l was achieved in this process. Although the mechanism through which the medium pH affected the product formation was not clear to them, they presumed some enzymatic regulations occurred in the

reductive conversion of acetyl-CoA to butyrate. It was inferred that the medium pH affects the performance of the involved enzyme directly by pH inhibition or indirectly through a pH-influenced mediator, possibly bicarbonate. To examine this hypothesis, similar batch fermentation was performed with a gas containing 80% CO and 20% CO_2 at pH of 6.8. CO_2 was used to increase the liquid phase concentration of bicarbonate, but the ratio of acetate to butyrate did not significantly change while adding CO_2. Thus, it was concluded that bicarbonate was not probably the primary metabolic regulator. On contrary, the results were consistent with direct pH regulation. For an equivalent amount of CO which is consumed during the fermentation process, acetate acid synthesis makes the medium more acidic that butyric acid, as it generated carboxyl groups 2.5 times more than that of butyric acid. Hence, at low pH values, butyrate production would be preferred to acetate to minimize further pH reduction.

Gaddy and Clausen [80] demonstrated that *C. ljungdahlii* produces acetate in preference to ethanol under optimum growth condition (pH: 5–7), while ethanol is favored over acetate under non-growth condition of pH: 4–4.5 and without yeast extract. Based on this knowledge, they utilized a two-stage continuous stirred tank bioreactor system with the aim of enhancing the ethanol production. The pH of the first reactor was set at 5 to promote the cell growth, then the pH was shifted to 4–4.5 and yeast extract was eliminated from the medium in the second reactor to cease the bacterial growth and onset ethanol production. As a result, with a gas stream containing 55.25% CO, 10.61% CO_2, 18.11% H_2 and 15.78% Ar, the ethanol concentration increased from 1 to 3 g/l and the ethanol to acetate molar ratio improved from 1 to 4 in the first and second bioreactor, respectively. These results indicated that the pH shift between the bioreactors and lack of yeast extract was a significant step to promote ethanol production at the expense of acetate.

Sakai et al. [34] isolated a thermophilic bacterium, *Moorella* sp. HUC22-1, from a mud sample collected from underground hot water which was able to produce ethanol from H_2 and CO_2 at 55 °C. A gas phase of 80% H_2 and 20% CO_2 was purged in serum bottles to a final pressure of 0.19 MPa. After cultivation time of 156 h, 1.5 mmol ethanol and 57 mmol acetate were produced. It was observed that accumulation of acetate in the culture caused a drastic decrease in pH and thus inhibited the cell growth and product formation. Thus, it was planned to control the culture pH in a fermentor connected to the gas reservoir. Control of pH at 6.2 resulted in production of 347 mmol acetate after 220 h, while ethanol production did not increase. Since the broth pH of 5 was suitable for ethanol production, the experiments initiated at the growth pH of 6.3, but controlled at 5 after initial pH decrease. In this pH-controlled batch culture, maximum ethanol production of 4.8 mmol was achieved. The cell growth, ethanol and acetate production in this culture was respectively 1.3, 3.7 and 2.4 times higher than that of pH uncontrolled batch culture.

6.4. Effect of reducing agent

Those bacteria which are unable to grow under high redox potential environment are defined as obligately anaerobic bacteria. Redox potential or oxidation/reduction potential is an indication of the tendency of a substance or solution to acquire electron and thus be reduced. In fermentation media, the redox potential is raised by oxygen that inhibits the growth of obligately anaerobic bacteria. Thus, lowering the redox potential of the growth medium is essential for the culture of anaerobic bacteria. For this purpose, reducing agent is added as an essential chemical component to the growth medium, to reduce the medium's redox potential. In fact, reducing agent is responsible to depress the redox potential and poise it at optimum values. Cysteine–HCl·H_2O, sodium sulfide ($Na_2S·9H_2O$), dithiothreitol, sodium thioglycolate, ascorbic acid, titanium (III)–citrate, potassium ferricyanide, methyl viologen and benzyl viologen are some of the commonly used reducing agents [17,101–103].

It has been reported that addition of reducing agent to the *Clostridium* fermentation media is effective in shifting the metabolism of bacteria toward solventogenesis. This is due to the availability of more reducing equivalents for the bacteria to form NADH for the conversion of acetyl-CoA to ethanol [17]. In fact, reducing agents alter the electron flow and direct the carbon flow to form ethanol over acid. Klasson et al. [35] assed the feasibility of improving the ethanol production over acetate in batch cultures of *C. ljungdahlii* containing small amount (30, 50 and 100 ppm) of various reducing agents (sodium thioglycolate, ascorbic acid, methyl viologen and benzyl viologen). Very limited growth was observed with 100 ppm of reducing agent in all cases. In contrast, the concentrations of 30 and 50 ppm successfully enhanced the ethanol to acetate ratio in most cases. The highest ethanol to acetate ratio of 1.1 was achieved with benzyl viologen at the concentration of 30 ppm, yielding in production of 3.7 mol of ethanol. They concluded that use of reducing agents which promote product ratio always cause the slower growth of bacteria due to the reduced ATP formation [35].

The optimum concentration of reducing agent is important to ensure a satisfactory level of redox potential in growth media for the anaerobic organism. Especially in the case of sodium sulfide, it is also important to note that sulfide added to the medium solution not only generates ions like HS^- or S^{2-}, but also can be converted to H_2S, depending on the solution pH. H_2S is a volatile compound with the potential to be lost during the experiment and thus changing the redox potential of the solution [103]. Therefore, understanding the fate of sulfide in the process and determining its optimum concentration is essential. In this regard, Sim and Kamaruddin [101] optimized the concentration of cysteine–HCl·H_2O (0.0–5.0 g/l) and $Na_2S·9H_2O$ (0.0–0.5 g/l) in the batch fermentation medium of *C. aceticum*. The obtained results showed that sodium sulfide did not exert considerable effect on cell growth and CO uptake rate, but it was required for the acetic acid production. Based on their optimization results, 0.3 g/l of each reducing agent was sufficient for 100% CO conversion and acetic acid production of 1.28 g/l within a fermentation time of 60 h. In another experiment carried out by Hu et al. [103] assessment the effect of sulfide (0.0–1.9 mM) was assessed during the course of syngas fermentation experiment using *Clostridial* bacteria denoted as P11. The results projected the positive effect of higher sulfide concentrations on ethanol production, while acetate formation was favored at lower sulfide concentrations.

As mentioned earlier, the growth of anaerobic bacteria can be stimulated in the presence of sulfur compounds due to their ability in reducing the redox potential of the medium. H_2S is one of the components usually found in syngas in trace amounts. Do et al. [97] reported that activity of CODH enzyme was stimulated in the presence of H_2S, while fermenting syngas using *R. rubrum*. It was discussed that CODH from *R. rubrum* is a redox sensitive enzyme whose activity is stimulated by H_2S which acts as a reducing agent to lower the redox potential of the fermentation medium.

6.5. Effect of trace metals

The acetogentic bacteria produce acetyl-CoA as a precursor for cell biomass production and formation of ethanol and acetate. The cell growth and product formation is improved by increasing the carbon flow to acetyl-CoA. To achieve this, the activity of the key metalloenzymes involved in the acetyl-CoA pathway including CODH, formate dehydrogenase and hydrogenase and alcohol dehydrogenase needs to be promoted. Trace metals available in

the growth medium can considerably affect the activity of these metalloenzymes which in turn result in improved cell growth and product formation. An investigation was undertaken by Saxena and Tanner [104] to study the effect of trace elements (Co^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Mo^{6+}, Ni^{2+}, Zn^{2+}, SeO_4^- and WO_4^-) on growth of *Clostridium ragsdalei* and the ethanol and acetate production by this acetogen. The result of this survey demonstrated that the presence of Cu^{2+} depressingly affected the ethanol production; however Ni^{2+} improved the cell growth and the activity of CODH and hydrogenase. The optimum concentrations of SeO_4^- and WO_4^- increased the activity of formate dehydrogenase, as tungsten and selenium has been suggested to act as cofactors in this enzyme. The cell growth and ethanol production was enhanced by increase of the Zn^{2+} concentration, but this increase did not affect the enzymes' activity. Omitting Fe^{2+} from the medium reduced the ethanol production and decreased the activity of the four enzymes, as all these metalloenzymes are iron–sulfur proteins.

Huhnke et al. [105] tested the effect of trace metal (Fe, Mo, Co, Ni and Cu) on CO metabolism during syngas fermentation using *C. ragsdalei*. Elimination of all metals, except Mo, improved the solvent to acid ratio, which was close to 300 and 400% for Fe and Ni. Elimination of Mo from the fermentation medium increased acetate production. However, Adams et al. [106] suggested Co limitation in the nutrient medium as a way to manipulate the biological pathway of acetogens to favor ethanol production over acetate. It was explained that Co limitation in the fermentation medium may reduce the acetyl-CoA cycle rate. Since Co is involved in transfer of a methyl group from THF cycle to acetyl-CoA cycle, limiting the Co concentration in the medium does not permit the transfer thus reducing the THF cycle function. Consequently, the ratio of NAD(P)H to NAD(P) is increased which improves ethanol production.

Lewis et al. [45] acknowledged that increasing the iron concentration in fermentation culture of *Clostridium carboxidivorans* to ten-fold compared to that in standard medium doubled the ethanol concentration, while reducing the acetic acid and butyric acid production. Elimination of iron from the standard medium inhibited the ethanol production. They examined the effect of trace metal on cell growth and product formation during the continuous fermentation of *C. carboxidivorans*, which is claimed as the first anaerobe able to carry out both direct and indirect fermentation of lignocellulosic biomass. They reported while doubling the trace metal concentration, the cell concentration almost doubled from 0.2 to 0.35 g/l. It was also observed that the CO utilization and ethanol, acetate and butanol production was promoted. Doubling the trace metals concentration for the second time during the continuous experiment resulted in initiation of cell death and termination of the experiment.

6.6. Inhibition in fermentation medium

In biological processes, the growth and/or product formation rate of microorganism may be reduced or even inhibited by reactants, products or contaminants. For example, production of organic acids is known to be associated with hydrogen formation. However, increase of the H_2 partial pressure in the gas phase or its accumulation in the fermentation medium might inhibit the fermentation and acetogenesis due to the alteration of carbon flow in the biological pathway of organism [73]. Also, in biological reactions, CO_2 can be a potential source of inhibition, as CO_2 affects the medium pH by formation of carbonic acid or its carbonate derivatives [57].

In a study to determine the optimum concentration of acetate in the batch fermentation medium of *R. rubrum*, the acetate concentration was varied in the range of 1–3 g/l [107]. Increase of the acetate concentration to 3 g/l, reduced the acetate conversion from 73 (at 1.5 g/l) to 23%, CO conversion from 97 (at 2 g/l) to 30% and H_2 production reduced by 50%. It was inferred that high acetate concentrations caused an inhibition in the batch media which retarded the microbial cell growth and negatively affected the H_2 production.

Ahmed et al. utilized *C. carboxidivorans* $P7^T$ [108] as a microbial catalyst for the conversion of biomass-generated syngas to ethanol and acetate. While introducing the biomass-generated syngas into the fermentation medium, it was observed that the microbial cell growth was dormant, H_2 consumption was inhibited and the product distribution between acetate and ethanol was altered. A likely scenario was that the tar available in the syngas caused the cell dormancy and product redistribution. Thus, a 0.025 mm filter was utilized in the sygas clean-up system to remove tar from the gas stream. However, it was observed that H_2 production was still inhibited [12]. This indicated that one of the syngas components inhibited the hydrogenase enzyme which is responsible for H_2 uptake. Generally, gases like O_2, CO, acetylene and nitric oxide (NO) are considered as hydrogenase inhibitor. It was hypothesized that 150 ppm NO detected in the biomass-based syngas was the possible cause of the hydrogenase inhibition. More investigation on this hypothesis revealed that NO at concentrations above 40 ppm was a non-competitive inhibitor of hydrogenase, however the loss of this activity was reversible. While the hydrogenase activity was inhibited by NO, the required electrons for ethanol production were provided from CO rather than H_2. This caused a reduction in the number of carbon available for product formation which consequently resulted in reduced carbon conversion efficiency. They concluded that the *C. carboxidivorans* cells could tolerate the NO content of the biomass-generated syngas at concentrations below 40 ppm, without any compromising the hydrogenase enzyme activity, microbial cell growth and product distribution.

Huhnke et al. [105] utilized fluoroacetate (FA) and trifluoroacetate (TFA) as metabolic inhibitors in fermentation culture of *C. ragsdalei* to inhibit acetogenesis to improve ethanol production relative to the cell growth yield. Use of 30 mM FA in carbon monoxide medium, decreased ethanol production, growth yield and acetate formation about 50, 40 and 90%, respectively, and the ethanol to acetate ratio improved 80%. While using 30 mM TFA in carbon monoxide medium, the ethanol and acetate increased by 126 and 52%, whereas the solvent to acid ratio improved only 50% and the cell growth remained unchanged. The obtained results indicated the shift of carbon flow from acidogenic to solventogenic phase for both utilized inhibitors.

6.7. Mass transfer limitations

Mass transfer of gas to liquid is typically the rate limiting step and potential bottleneck in syngas fermentation. This limitation is expected to be more sever in syngas fermentation in comparison to aerobic process, as the solubility of CO and H_2 is 60 and 4% (on a mass basis) of that of oxygen [39]. Mass transfer limitation may originate from either of the following steps: the transfer of the gaseous substrate into gas–liquid interface; transport in aqueous fermentation media; diffusion of the gas into the liquid layer surrounded the microbe and diffusion of the gaseous substrate through the microbial envelope to the intracellular reaction site. For the sparingly soluble gases, the transfer to the liquid media through the gas–liquid interface is the major mass transfer resistance [35,90,109].

The bioreactor for gaseous system should be operated in either of two regimes. In the first regime, there are sufficient cells in the aqueous phase to consume the solute, however the mass transfer rate cannot be kept in pace with the cell demand. Although the reaction rate may be inherently fast, but sufficient substrate is not supplied fast enough to promote the overall reaction rate. Hence,

the liquid concentration of solute is nearly zero and the reaction is mass transfer limited. In this case, the cell mass and reaction rate is restricted by the ability of the bioreactor to transfer the gaseous substrate [35,57]. In the second regime, although sufficient substrate is provided in the aqueous phase, however the cell concentration is not high enough to consume the substrate. This is the case in which the reaction rate is limited by the cell concentration; the liquid phase concentration of the gaseous substrate is not zero which increases the possibility of substrate inhibition. Thus, the bioreactor should be designed and operated in a way that high cell concentration and enhanced mass transfer rate would be attainable [35].

Generally, the mass transfer rate of the gaseous substrate into the liquid fermentation media is enhanced by increasing the gas solubility in the media or reducing the mass transfer resistance at the gas–liquid interface by reducing the surface tension [23]. In suspended growth bioreactors, the gas bobble diameter is a key factor in gas–liquid mass transfer. The specific surface area available for mass transfer and gas bubble diameter are in reverse relation under mass transfer limited condition. Dispersing the sparingly soluble gaseous substrate in the fermentation medium would provide a high surface area for mass transfer. Also, reducing the gas bubble size during the fermentation process would cause the slow rise of the bubble which results in longer gas hold-up time in the bioreactor [17]. The effectiveness of the mass transfer rate among various reactor configurations is usually compared using the gas–liquid volumetric mass transfer coefficient (K_La) which corresponds to the hydrodynamic condition in the reactor [110]. In this regard, stirred tank reactors are the most widely implemented approach to improve the mass transfer coefficient (K_La). Use of impeller in stirred tank reactors generates a hydrodynamic shear which breaks up large bubbles into smaller size and thus increases the interfacial surface area available for mass transfer [39]. Increase of gas velocity can also improve K_La, however the high gas flow rate may adversely affect the substrate gas conversion. This reduced conversion efficiency is enhanced by gas recycling [39].

Continuous hydrogen production from syngas by *R. rubrum* was demonstrated in a 2l stirred tank bioreactor by Younesi et al. [25]. The effect of agitation speed (150–500 rpm) and gas flow rate (5–14 ml/min) on hydrogen production was monitored during a period of 60 days. The obtained results revealed the improvement of H_2 production and mass transfer coefficient with increase of the agitation speed and gas flow rate. However, the CO conversion dropped from 95 to 87% as the gas flow rate was raised from 5 to 14 ml/min. Maximum H_2 production rate and yield of 16 mmol/g h and 80% were achieved at agitation speed of 500 rpm and gas flow rate of 14 ml/min. At this condition, the mass transfer coefficient (K_La) of 72.8 h^{-1} was obtained. In another investigation conducted by Ismail et al. [63] the effect of agitation speed (350–800 rmp) on K_La and hydrogen production by *R. rubrum* was studied. Although increase of the agitation speed from 350 to 800 rpm promoted the mass transfer coefficient from 52.2 to 162.2 h^{-1} and improved the hydrogen production from 5.3 to 12 mmol/l, however foaming was observed as the major concern at agitation speeds of more than 700 rpm. It was inferred that this undesirable phenomenon caused a reduction in H_2 production and K_La after 48 h of operation, probably due to the decrease of working volume and accumulation of reactants or products in foam. They also reported that sparging the syngas into the fermentation medium through a microsparger created some microbubbles which increased the H_2 production 15–40% in comparison to the simple ring sparger.

The study of the hydrodynamics of stirred bioreactors has led to some advancement in impeller design which could be useful in modifying the flow patterns, power efficiency and mixing time. In this aspect, Ungerman and Heindel [109] implemented eleven different impeller schemes in a stirred tank bioreactor and examined their effect on K_La and power demand in CO fermentation. The result of this study showed that the highest K_La was obtained with dual Rushton-type impeller; however this scheme exhibited high power consumption. They concluded that K_La values close to those of dual Rushton-type impeller with reasonable power consumption can be achieved with dual impeller schemes with low concave impeller, especially at high gas flow rates.

Although increasing the agitation speed increases the bubble break up and improves the mass transfer of the slightly soluble gases; however use of very high agitation speeds and vigorous mixing generate excessive shear which is known to damage the shear-sensitive microorganisms and lead to loss of viability and cell disruption [63,109]. The more important issue is that high input power is not beneficial from economical point of view for commercial syngas fermentation plants. In contrast, trickle-bed reactors and bubble column reactors which consider as columnar reactor do not require mechanical agitation which may decrease their power cost.

Trickle-bed reactors may be operated in gas-continuous or liquid-continuous mode. In gas-continuous reactors, there is a low mass transfer resistance is gas side for sparingly soluble gases. Thus, increase of the gas flow rate does not considerably affect the mass transfer coefficient (K_L) and specific surface area (a). For this reason, the gas flow rate may be kept at the lowest possible value to achieve high conversion efficiencies, without a considerable loss in K_La. Conversely, in liquid-continuous reactors, the gas flow rate strongly affects the interfacial surface area. In this case, to obtain a high K_La, the gas velocity should be increased, but gas recirculation may be required to maintain high conversion efficiencies [39].

In bubble column reactor a high mass transfer rate is achieved and low operation and maintenance costs are considered as the primary advantages of this reactor, however drawbacks such as back mixing and coalescence are the major issues in this system. Operationally, there is an upper limit for increasing the flow rate, beyond which a heterogeneous flow is created which eventually, causes the back mixing of the gas components [17,90].

Klasson et al. (methane production, Performance of Trickle-) utilized a mixed-culture containing the photosynthetic bacterium, *R. rubrum*, to convert CO to CO_2 and H_2 through the water–gas shift reaction followed by conversion of CO_2 and H_2 to CH_4 using two methanogens, *Methanobacterium formicicum* and *Methanobacterium barkeri*. Two types of bioreactors, a packed bubble column and a trickle-bed reactor, were implemented for tri-culture operation and the results were compared. A mixed culture of two methanogens was employed because the bacterium *M. formicicum*, shows a high H_2 uptake rate, but is inhibited in the presence of CO, whereas, the other bacterium, *M. barkeri*, expresses higher CO tolerance, but converts H_2 in a lower rate. While fermenting a gas stream containing 55% CO, 20% H_2, 10% CO_2 and 15% Ar, a product yield ($Y_{CH4/H2}$) of 0.34 and 0.2 was achieved in packed bubble column and trickle-bed reactor, respectively. It was claimed that the yield obtained in the packed bubble column using the mix-culture was 36% higher than the theoretical yield. A CO conversion of 100% was attained in the trickle-bed reactor, whereas a conversion of higher than 79% was not achievable in the packed bubble column. The higher conversions obtained in the trickle-bed reactor was justified by the longer gas residence time and improved mass transfer properties as a result of lower liquid porosity (ε_L) encountered in this reactor in comparison to the packed bubble column. Also, a considerably higher productivity (3.4 mmol CH_4/l h) was demonstrated using the trickle-bed reactor compared to the packed bubble column (0.4 mmol CH_4/l h). The trickle-bed reactor showed to provide a much higher mass transfer coefficient, as the K_La values of 780 h^{-1} and 3.5 h^{-1} were achieved for the trickle-bed reactor and the packed bubble column, respectively.

Immobilized cell reactor (ICR) offers the advantages of high cell densities and plug flow behavior. The high surface to volume ratios provided in the reactor promotes the mass transfer rate with reduced back mixing. In this bioreactor, the direct contact of microbe with gaseous substrate minimizes the diffusion resistance. Also, high cell densities achieved in this reactor reduces the retention time. However, the overgrowth of the cells can completely fill the interstitial spaces and cause severe channeling problems. The ICRs are not flexible enough to be used in various operation conditions as the design of bioreactor is limited to the column dimensions and packing and thus the contacting capability of the bioreactor is somehow fixed. This is considered as the major disadvantage of ICRs [35].

Use of chemicals which dissolve in the liquid phase, show low toxicity and reduce surface tension to increase the overall mass transfer rate could be also useful to improve the gas–liquid mass transfer. Several chemicals including bio-polymers (xanthan gum, dextran), bio-surfactant (bio-detergents) and organic compounds (high carbon alcohols, perfluorocarbon compounds) were used for this purpose [23]. Gaddy and Chen [23] investigated the effect of various co-solvents and surfactants on mass transfer in a batch culture of anaerobic bacterium, *B. simithii* ERIH2. Addition of 0.1 vol.% of the detergents TRITON N-101^{TM}, NONIDET P-40^{TM}, Triton X-100 and TYLOXAPOL^{TM} improved the mass transfer coefficient, K_La, by 84%, 107%, 203% and 340%, respectively compared to the control. As they concluded, foaming during this study was an indication of the positive effect of bio-detergents in reducing the surface tension rather than improving the solubility of CO in the liquid media.

It has also been demonstrated that addition of small particles or catalysts to the fermentation medium can enhance the gas liquid mass transfer. With the intention of improving K_La, Zhu et al. [111] synthesized spherical MCM41 nanoparticles and grafted them to various functional groups like methyl, nitrilpropyl, carboxylpropyl and mercaptopropyl. The obtained results indicated that the hydroxyl group on nanoparticles enhanced the CO mass transfer due to the absorption of CO from gas bubble by this group and then its release in water. In another work [112], they implemented the fabricated nanoparticles during the batch fermentation of syngas (48.2%CO, 31.8%H_2 and 20% CO_2) to H_2 using *R. rubrum*. It was observed that utilization of 0.6 wt% of the MCM41 nanoparticles functionalized with mercaptopropyl group improved H_2 yield by about 200%. Such enhancement in H_2 yield was resulted from the promoted CO mass transfer by addition of MCM41 nanoparticles.

6.8. Effect of substrate pressure

In the batch fermentation, at a certain level of cell density, utilization of sparingly soluble gases such as CO and H_2 becomes mass transfer limited in shaken bottles. In this case, the volumetric uptake of the gaseous substrate is proportional to the partial pressure of that component in the gas phase [113]. The partial pressure of gas components can significantly affect the microbial growth and product distribution, as the enzymes involved in the metabolic process are sensitive to substrate exposure [114]. The reaction rate also increases linearly with respect to the gas pressure which results in a reduction in effective retention time. The volume of reactor can also be reduced, while using high pressure gaseous substrate [22].

As mentioned before, in the absence of H_2 in gaseous substrate, CO can act as an electron donor via CODH. Hence, an increase in the partial pressure of CO improves its net electron production by CODH. Nevertheless, in the case of organisms which are less resistant to CO, prolonged doubling time has been observed while increasing the CO partial pressure [17]. Sim et al. [76] studied the batch conversion of CO to acetic acid using *C. aceticum* under various CO partial pressures of 1.4–2.02 atm. It was observed that *C. aceticum* had a high CO tolerance and high CO pressures did not inhibit the cell growth. Although the highest cell concentration of 893.63 mg/l was achieved at CO partial pressure of 2.02 atm, however, this pressure did not lead to the highest CO consumption and acetic acid production. It was deduced that this situation was probably attributed to the cell lysis due to the reduction of CO transfer from the gas phase into the liquid, because of the reduced driving force. At the CO partial pressure of 1.55 atm, maximum acetic acid production of 2.27 g/l and CO conversion of 100% was achieved within 72 h. In another study carried out by Gaddy and Chen [23], *R. rubrum* was grown under various CO partial pressure of 0.8–1.4 atm. They reported that the effect of the partial pressure on CO uptake rate, cell growth and H_2 yield was not appreciable even at pressures up to 1.4 atm.

Citrobacter sp. Y19 was found as a facultative anaerobe whose growth was observed to be much faster under aerobic condition [26]. However, H_2 evolution was observed only under anaerobic conditions. In an attempt to investigate the effect of CO partial pressure on H_2 production using this bacterium, Jung et al. [59] increased the CO partial pressure in the gas phase from 0.0 to 0.5 atm, in the batch experiments. It was found that the CO partial pressure of 0.05 atm was the proper pressure for optimum cell growth and H_2 production, beyond which the H_2 production activity and specific growth rate decreased. It was discussed that the growth of anaerobic bacteria is little affected by CO. However, in the case of aerobic organisms, the microbial growth is inhibited by CO due to the interference of CO with electron transport chain. It was suggested that CO prevents cytochrome oxidase from coupling with oxygen in the electron transport system.

Klasson et al. [35], examined the effect of initial CO partial pressure on CO uptake rate of *Peptostreptococcus productus*. There was a linear relation between the reaction rate and CO partial pressures up to 1.6 atm. At CO partial pressure of 2.5 atm, there was a short period of CO uptake, thereafter the culture failed to utilize the CO. This was probably due to the insufficient cell concentration which could not keep the reaction at the mass transfer limited stage and caused toxicity for the microbe. It was suggested that high CO partial pressure could be employed after a sufficient cell concentration was achieved. They used a gradual stepwise procedure to increase the CO partial pressure, after a high cell concentration was achieved. This way, they successfully increased the CO partial pressure to 10 atm.

The effect of CO partial pressure on cell growth and ethanol and acetate production was also assessed by Hursta and Lewis in batch fermentation of *C. carboxidivorans*. Increase of the CO partial pressure from 0.35 to 2.0 atm, increased the cell concentration from 0.20 to 1.08 g/l, which represented a 440% improvement. Acetate was found as a growth-associated product which generates ATP for cell growth and its production was almost similar and constant in all cases. The graph obtained for ethanol production versus cell dry weight showed a transition from non-growth-associated to growth-associated ethanol production, along with increase of the CO partial pressure. Ethanol production also initiated at earlier times with increase of the CO partial pressure. Younesi et al. [84,115] also studied the effect of various initial total pressures of syngas (0.8–1.8 atm) in the batch culture of *C. ljungdahlii*. The result of this investigation indicated that the cell population was not inhibited even at high syngas pressures of 1.6 and 1.8 atm, however the cell concentration as well as acetate concentration was almost the same in all utilized syngas total pressures. Ethanol production was promoted at high pressures. Such increase was probably attributed to the utilization of CO_2/H_2, beside CO as the gaseous substrate for ethanol production and improved mass transfer rate at elevated pressures.

7. Process scale up and commercialization

Since the beginning of the 20th century, various strategies to produce fuels and chemicals from syngas have been under investigation and development [91]. However, most of this exploration has been focused on the deployment of metal-based catalytic process. Recently, special attentions have been paid to the conversion of syngas to biofuels and biochemicals through the microbial routes due to the preferences proposed for biocatalysts over metal-based catalysts. Currently, several syngas fermentation approaches are under evaluation in research laboratories and pilot plants. In spite of this increased interest, syngas fermentation development plants are still at the pre-commercial stage and various technical and economic challenges should be obviated before the commercial scale plants could be established.

Generally, slow reaction rate and the need for sterile condition to prevent media contamination are some of the disadvantages involved in biological process. However, in process of syngas fermentation, presence of CO in gas stream ensures sterility as it is toxic to most microorganisms. Mass transfer limitation is another issue involved in biological process due to the slight solubility of the gaseous substrate especially CO and H_2 in fermentation broth [51]. So far, a limited range of chemicals, mainly acetate and ethanol, have been produced from syngas through fermentative process and the known organisms are not efficient producers of other chemicals and genetic manipulation may be required to improve the efficiency of the organisms as well as their sensitivity to the high concentrations of end products [15,92]. Due to these barriers commercial deployment of syngas fermentation plants still face major constraints.

Although so far just three major companies of INEOS Bio (USA, 2008), Coskata (USA, 2009) and LanzaTech (New Zealand, 2010) have reported the successful operation of large facilities for high ethanol production via syngas fermentation [91,92], however the fermentation of syngas would likely be considered as one of the potential options for second-generation fuels production for many years to come.

8. Conclusion

Promotion of biofuels generated from syngas bioconversion can provide solutions for the energy security issues and contribute to the greenhouse gases mitigation strategies. The high potential of microbial catalyst to convert syngas and waste gas substrates to valuable biofuels and biochemicals have been confirmed by various successful researches and patent literatures. This tends to reinforce the suitability of the microbial catalysts to carry out the syngas conversion due to their inherent merits. Despite years of research and recent progress, significant efforts should continue to be made toward making the process cost effective and reliable for large scale deployments.

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